CLAIMS

1. A ligand profile which is characteristic for a given cell, the ligand profile comprising a representation of at least ten different polypeptide ligands, all of which bind to a single type of multi-ligand binding receptor, wherein the representation characterizes each individual ligand based upon at least three physical or chemical attributes; provided that, if the multi-ligand binding receptor is an MHC class I or class II receptor, at least 500 polypeptide ligands are represented in the ligand profile; and further provided that the ligand profile is a reproducible characteristic of the cell.

2. A ligand profile which is characteristic for a given cell, the ligand profile comprising a representation of at least ten different polypeptide ligands, all of which bind to a single type of multi-ligand binding receptor, wherein the representation characterizes each individual ligand based upon at least two physical or chemical attributes, one of said attributes being mass or mass-to-charge ratio; provided that, if the multi-ligand binding receptor is an MHC class I or class II receptor, at least 500 polypeptide ligands are represented in the ligand profile; and further provided that the ligand profile is a reproducible characteristic of the cell.

3. A ligand profile which is characteristic for a given cell, the ligand profile comprising a representation of at least ten different polypeptide ligands, all of which bind to a single type of multi-ligand binding receptor, wherein the representation characterizes each individual ligand based upon at least one physical or chemical attribute, the at least one physical or chemical attribute

comprising amino acid sequence; provided that, if the multi-ligand binding receptor is an MHC class I or class II receptor, at least 50 polypeptide ligands are represented in the ligand profile; and further provided that the ligand profile is a reproducible characteristic of the cell.

4. A ligand profile which is characteristic for a given cell, the ligand profile comprising ion fragmentation patterns for at least ten different polypeptide ligands, all of which polypeptide ligands bind to a single type of multi-ligand binding receptor; provided that, if the multi-ligand binding receptor is an MHC class I or class II receptor, at least 100 polypeptide ligands are represented in the ligand profile; and further provided that the ligand profile is a reproducible characteristic of the cell.

5. A ligand profile which is characteristic for a given cell, the ligand profile comprising amino acid sequences of at least ten different polypeptide ligands having distinct core peptides, all of which ligands bind to a single type of multi-ligand binding receptor; provided that, if the multi-ligand binding receptor is an MHC class I or class II receptor, at least 100 polypeptide ligands are represented in the ligand profile; and further provided that the ligand profile is a reproducible characteristic of the cell.

6. The ligand profile of claim 1, wherein the multi-ligand binding receptor is an MHC class I or MHC class II receptor.

7. The ligand profile of claim 1, wherein the multi-ligand binding receptor is not an MHC class I or MHC class II receptor.

8. The ligand profile of claim 1, wherein the multi-ligand binding receptor is a chaperone, a chaperonin, a calnexin, a calreticutin, a mannosidase, a N-glycanase, a BIP, a grp94, a grp96, hsp60, hsp65, hsp70, hsp90, hsp25, an E2 ubiquitin carrier protein, an E3 ubiquitin ligase, an unfoldase, hsp100, a proteasome, a trafficking protein, or a retention protein.

9. The ligand profile of claim 1, combined with a second ligand profile, the second ligand profile (a) also being a reproducible characteristic of the given cell, and (b) comprising a representation of at least ten additional polypeptide ligands, all of which bind to a second type of multi-ligand binding receptor different from the first type of receptor.

10. A method of generating a reproducible ligand profile for a given cell type, which cell type comprises a selected type of multi-ligand binding receptor, the method comprising:

(a) providing a first sample of the given cell type, wherein the first sample comprises a first plurality of polypeptide ligands bound to the selected type of multi-ligand binding receptor;

(b) isolating the selected type of multi-ligand binding receptor from the first sample;

(c) separating the first plurality of ligands from the selected type of multi-ligand binding receptor;

(d) fractionating the first plurality of ligands;

(e) generating a first profile distinguishing among the first plurality of ligands on the basis of at least one chemical or physical attribute;

(f) providing a second sample of the given cell type, the second sample being essentially identical to the first sample, wherein the second sample comprises a second plurality of polypeptide ligands bound to the selected type of multi-ligand binding receptor;

(g) isolating the selected type of multi-ligand binding receptor from the second sample;

(h) separating the second plurality of ligands from the selected type of multi-ligand binding receptor;

(i) fractionating the second plurality of ligands;

(j) generating a second profile distinguishing among the second plurality of ligands on the basis of the at least one chemical or physical attribute; and

(k) confirming that the first profile and the second profile are essentially identical, and together represent a reproducible ligand profile for the given cell type.

11. The method of claim 10, wherein a second chemical or physical attribute of each ligand is determined subsequent to the fractionation steps, and is represented in the profiles.

12. The method of claim 11, wherein a third chemical or physical attribute of each ligand is determined subsequent to the fractionation steps, and is represented in the profiles.

13. The method of claim 10, wherein the isolating and separating steps are accomplished using appropriate columns arranged in an in-line system.

14. A method of generating a ligand profile for a given type of cell, comprising:

(a) providing a sample of lysate of the given type of cell, wherein the sample comprises a first plurality of polypeptide ligands bound to a first type of multi-ligand binding receptor and a second plurality of polypeptide ligands bound to a second type of multi-ligand binding receptor;

(b) isolating the first and second types of multi-ligand binding receptors from the sample;

(c) separating the first plurality of ligands from the first type of multi-ligand binding receptor and the second plurality of ligands from the second type of multi-ligand binding receptor;

(d) fractionating the first plurality of ligands and the second plurality of ligands; and

(e) generating a first profile distinguishing among the first plurality of ligands on the basis of at least one chemical or physical attribute and a second profile distinguishing among the second plurality of ligands on the basis of the same at least one chemical or physical attribute.

15. A method of generating a subtraction profile of polypeptide ligands, comprising:

(a) producing a first ligand profile by a method comprising:

(i) providing a first sample comprising a first cell of interest, wherein the first cell of interest

comprises a given type of multi-ligand binding receptor bound to a first set of polypeptide ligands;

(ii) isolating the given type of multi-ligand binding receptor and the first set of ligands from the first sample;

(iii) separating the first set of ligands from the given type of multi-ligand binding receptor;

(iv) generating a first profile distinguishing among the first set of ligands on the basis of at least one chemical or physical attribute;

(b) producing a second profile of ligands by a method comprising:

(i) providing a second sample comprising a second cell of interest, wherein the second cell of interest comprises the given type of multi-ligand binding receptor, bound to a second set of polypeptide ligands;

(ii) isolating the given type of multi-ligand binding receptor and the second set of ligands from the second sample;

(iii) separating the second set of ligands from the given type of multi-ligand binding receptor;

(iv) generating a second profile distinguishing among the second set of ligands on the basis of the same at least one chemical or physical attribute;

(c) comparing the first profile and the second profile to identify differentially expressed ligands, thereby forming a subtraction profile of ligands.

16. A subtraction profile generated by the method of claim 15.

17. A method of comparing a first cell sample to a reference cell sample, comprising:

(a) producing a first ligand profile by a method comprising:

(i) providing a first cell sample comprising a given type of multi-ligand binding receptor bound to a first set of polypeptide ligands;

(ii) isolating the given type of multi-ligand binding receptor and the first set of ligands from the first cell sample;

(iii) separating the first set of ligands from the given type of multi-ligand binding receptor;

(iv) generating a first ligand profile distinguishing among the first set of ligands on the basis of at least one chemical or physical attribute;

(b) providing a reference ligand profile representing a second set of polypeptide ligands extracted from the given type of multi-ligand binding receptor of a reference cell sample, wherein the reference ligand profile distinguishes among the second set of polypeptide ligands on the basis of the at least one chemical or physical attribute; and

(c) comparing the first ligand profile to the reference ligand profile, in order to identify differences or similarities between the first cell sample and the reference cell sample.

18. The method of claim 17, wherein the reference cell sample consists essentially of healthy cells of an animal and the first cell sample comprises cells suspected of being diseased.

19. The method of claim 17, wherein the first cell sample comprises cells cultured in the presence of a test compound, and the reference cell sample does not.

20. The method of claim 17, wherein the reference cell sample comprises cells cultured in the presence of a test compound, and the first cell sample does not.

21. A set of ligand profiles, comprising

(a) a first ligand profile comprising a first representation of a first plurality of polypeptide ligands, all of which bind to at least one multi-ligand binding receptor of a first cell, wherein the first representation distinguishes among the members of the first plurality of ligands based upon at least one physical or chemical attribute; and

(b) a second ligand profile comprising a second representation of a second plurality of polypeptide ligands, all of which bind to the at least one type of multi-ligand binding receptor of a second cell, wherein the second representation distinguishes among the second plurality of ligands based upon the at least one physical or chemical attribute;

provided that (i) the first cell differs from the second cell in a parameter selected from the group consisting of genetic background, culture conditions, genetic background plus culture conditions, *in vivo* exposure to a test compound, and genetic background plus *in vivo* exposure to a test compound; and (ii) any significant difference between the first and the second ligand profiles is attributable to that parameter.

22. A method of detecting a difference between the set of proteins expressed in a first cell and the set of proteins expressed in a second cell, comprising

(a) providing a first ligand profile made by a method comprising

(i) providing a first cell comprising at least one type of multi-ligand binding receptor, bound to a first set of polypeptide ligands,

(ii) isolating from the first cell the at least one type of multi-ligand binding receptor bound to the first set of ligands,

(iii) separating the first set of ligands from the at least one type of multi-ligand binding receptor, and

(iv) generating a first ligand profile distinguishing among the members of the first set of ligands on the basis of at least one chemical or physical attribute;

(b) providing a second ligand profile made by a method comprising

(i) providing a second cell comprising the at least one type of multi-ligand binding receptor, bound to a second set of polypeptide ligands,

(ii) isolating from the second cell the at least one type of multi-ligand binding receptor, bound to the second set of ligands,

(iii) separating the second set of ligands from the at least on type of multi-ligand binding receptor, and

(iv) generating a second ligand profile distinguishing among the members of the second set of ligands on the basis of the at least one chemical or physical attribute;

(c) comparing the first ligand profile to the second ligand profile, in order to identify any difference between the first and second profiles, wherein such a difference is an indication of a difference between the set of proteins expressed in the first cell and the set of proteins expressed in the second cell.

23. The method of claim 22, comprising the further step of

(d) generating a differential profile which sets forth at least some of the differences between the set of proteins expressed in the first cell and the set of proteins expressed in the second cell.

24. A differential profile generated by the method of claim 23.

25. The method of claim 22, comprising the further steps of selecting a ligand which is represented in one profile but not in the other, and identifying the amino acid sequence of the ligand.

26. A database, stored on a machine-readable medium, comprising

three categories of data respectively representing (a) ligand profiles, (b) cell sources, and (c) receptor types, and

associations among instances of the three categories of data,

wherein the database configures a computer to enable finding instances of data of one of the categories based on their associations with instances of data of another one of the categories.

27. The database of claim 26 in which data representing the cell sources comprise data identifying at least one type of cell.

28. The database of claim 26 in which data representing the cell sources comprise data identifying at least one cell condition.

29. The database of claim 26 in which data representing the cell sources comprise data identifying at least one individual animal.

30. The database of claim 26 in which data representing the cell sources comprise data identifying at least one state of perturbation.

31. The database of claim 26 in which data representing the cell sources comprise data identifying at least one developmental state.

32. The database of claim 26 in which the ligand profiles comprise information that uniquely identifies protein fragments.

33. The database of claim 26 in which the ligand profiles comprise mass spectral data.

34. The database of claim 26 in which the database configures the computer to enable finding at least one instance of the ligand profiles that is associated with a selected one or more instances of the cell sources and a selected one or more instances of the receptor types.

35. A machine-implemented method comprising

forming a query for searching a database, the database comprising three categories of data respectively representing (a) ligand profiles, (b) cell sources, and (c)

receptor types, the database defining associations among instances of the three categories of data, the query comprising one or more instances of one of the three categories of data, and

applying the query to the database to find instances of another one of the three categories of data.

36. The method of claim 35 in which the found instances comprise two ligand profiles.

37. The method of claim 36 further comprising

comparing the two ligand profiles to determine a difference between them.

38. The method of claim 36 in which the query comprises instances of a selected cell source comprising a selected cell condition.

39. A machine-based method comprising

performing an experiment on cells,

identifying a ligand profile associated with said cells, and

based on the ligand profile, querying a database that contains at least two categories of data, including ligand profiles and cell sources, to derive a cell source or a ligand profile and an associated cell source.

40. The method of claim 39 in which

the feature of the experiment comprises treatment of the cells using a candidate drug regimen, and

a cell source identified as a result of the query represents a different treatment of cells.

41. A machine-assisted method of investigation comprising

identifying a cell source, a receptor type, or a ligand profile of interest, and

based on the identified cell source, receptor type, or ligand profile, querying a database that contains three associated categories of data respectively representing (a) ligand profiles, (b) cell sources, and (c) receptor types, to derive information about cell sources, receptor types, or ligand profiles that relates to the cell source, receptor type, or ligand profile of interest.

42. A machine-assisted method comprising

providing cells of a cell source,

generating a ligand profile from the cells, and

based on the ligand profile and the cell source, querying a database that contains three associated categories of data respectively representing (a) ligand profiles, (b) cell sources, and (c) receptor types, to derive information about cell sources, receptor types, or ligand profiles that relates to the provided cell source and the generated ligand profile.